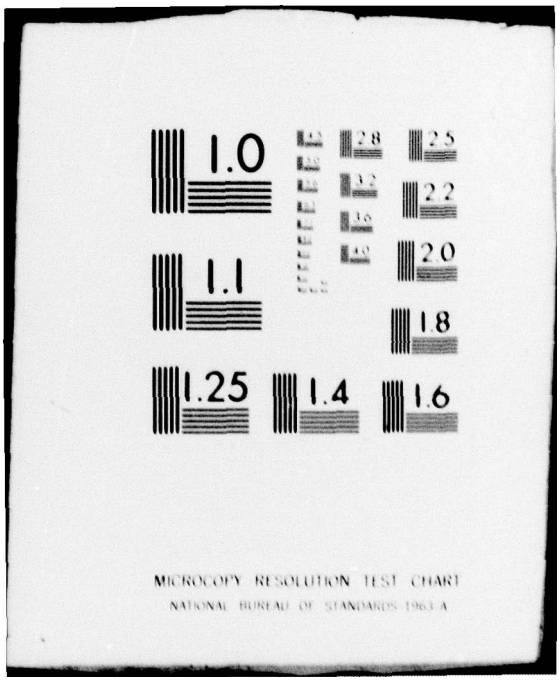
AD-A074 565 BIOMEDICAL RESEARCH INST ROCKVILLE MD F/G 6/15
SCHISTOSOME MATERIALS FOR VACCINE DEVELOPMENT.(U)
SEP 79 M A STIREWALT, F A LEWIS N00014-76-C-0146

UNCLASSIFIED NL

| OF |
AD A074565

END
DATE FILMED
10-79
DDC


1.0
1.1
1.25
1.4
1.6
2.8
2.5
3.2
2.2
3.6
2.0
4.0
1.8
MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS 1963 A

AD A074565

LEVEL II

SEP 24 1979

File: NR204-005 Stirewalt.

(1)

(9) Annual rept. no. 4, 30 Sep 78 – 1 Oct 79,

Office of Naval Research

(15) Contract ~~#ONR~~ N00014-76-C-0146

Task No. NR 204-005

Annual Report No. 4

(6) Schistosome Materials for Vaccine Development

by

(10) M. A. Stirewalt

~~and~~

F. A. Lewis

(12) 10

(11) 24 September 1979

DDC RECEIVED OCT 2 1979 A

Reproduction in whole or in part is permitted for any purpose of the United States Goverment

DDC FILE COPY

DISTRIBUTION STATEMENT A
Approved for public release;
Distribution Unlimited

79 10 01 096

388 117

JOB

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER: 4	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle): SCHISTOSOME MATERIALS FOR VACCINE DEVELOPMENT		5. TYPE OF REPORT & PERIOD COVERED: Annual: 30 Sep 1978- 1 Oct 1979
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s): M. A. Stirewalt and F. A. Lewis		8. CONTRACT OR GRANT NUMBER(s): ONR N00014-76-C-0146
9. PERFORMING ORGANIZATION NAME AND ADDRESS: American Foundation for Biological Research 12111 Parklawn Drive Rockville, Maryland 20852		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS: NR 204-005
11. CONTROLLING OFFICE NAME AND ADDRESS: Procurement Contract Officer Office of Naval Research (443) Dept. of the Navy Washington, D.C. 20360		12. REPORT DATE: 24 Sep 1979
		13. NUMBER OF PAGES: 8
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		15. SECURITY CLASS. (of this report): Unclassified
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Standard Distribution Lists

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Schistosoma mansoni; trematode; cercariae; worms; penetration enzymes; rotifer inhibition; optimal maintenance conditions.

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Temperature and food being controlled at optimal levels, the presence of rotifers in the snail colonies was the most important factor in parasite maintenance. Rotifer infestation of schistosome-infected snails was highly inimical to parasite development. With rotifer control, large numbers of schistosomes and products of host-shistosome interaction were produced on a weekly schedule. Limits of variability in production of parasites were set for the laboratory conditions.

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

S/N 0102-LF-014-6601

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

BACKGROUND

The Immunoparasitology Department at the Naval Medical Research Institute (NMRI) is involved in research centered primarily on the development of effective vaccines against several parasitic diseases. Immunological research in one such disease, schistosomiasis, is particularly difficult due to the limited quantity of schistosomal materials available to most laboratories. It has been the objective of this contract to supply large quantities of schistosomal materials to investigators at NMRI and the Biomedical Research Institute (BRI) to help realize the goal for the development of an effective vaccine against schistosomiasis. The various materials provided include adult schistosomes, eggs, cercariae, schistosomules, cercarial penetration enzymes, and vertebrate and invertebrate host serum and tissues.

Accession For
NTIS GRA&I
DDC TAB
Unannounced
Justification
By
Distribution/
Availability Codes
Dist. | Avail and/or special
A

METHODOLOGY

A Puerto Rican strain of Schistosoma mansoni was maintained in Biomphalaria glabrata snails and Swiss albino mice. Six 20-gallon aquaria, supporting approximately 1000 snails each, provided uninfected snails. Approximately 300 snails (5-7mm dia.) were collected each week and exposed individually with 6-8 miracida. When requested, snails were exposed to 1 miracidium each for development of single-sex schistosomal infections. Miracidia were derived from livers of 8-week infected mice. A constant supply of approximately 1000 infected snails were maintained for production of cercariae.

From these infected snails, from 1 to 3 million cercariae were collected 4 days per week and processed as needed or used for experimental work.

For the production of adult parasites and eggs two hundred and fifty Swiss mice were exposed percutaneously weekly to 250-270 cercariae each. Adult worms were perfused from these mice at 7 weeks post-infection, and eggs isolated from the livers. Schistosomules, the postpenetration stage of cercariae, were recovered from ear skin of mice exposed in vivo to cercariae. Schistosomules were also collected after cercarial penetration of dried rat abdominal epidermis, or by the Colley syringe shear technique.

Fifty to 100 ml of secreted enzyme solution were harvested two to four days each week from cercariae stimulated to secrete in a temperature gradient over skin surface lipid or the active fraction, linolenic acid. Total protein (Lowry method) and enzyme activity against azocoll (dye-coupled collagen) were established spectrophotometrically for each collection.

RESULTS AND DISCUSSION

The list of investigators at NMRI and BRI for whom schistosomal materials were supplied by the contract is given in Appendix 1. Each week the materials supplied consisted of: 8 to 12 million cercariae; 20 to 25 thousand adult worms; 600,000 to 900,000 schistosomules, the numbers adjusted to demand; frozen irradiated schistosomules; about 200ml of preacetabular gland enzyme secretion; and excretions and secretions of cercariae as requested.

Maintenance of this large-scale life cycle of S. mansoni allowed investigations into the nature of the observed natural fluctuations encountered in cercarial harvests and infectivity. We noticed that cercarial production decreased during infestation of the snail colony with rotifers of the genera Philodina and Rotifer. A dramatic decrease in cercarial production per snail (Figure 1) was noted, as well as an inhibition of normal cercarial activity. The cercariae which did emerge from the affected snails were lethargic, accumulated on the bottom surface of the dish, and had tightly curled tail furci.

In summary, the provision of schistosomal materials on a large scale requires close control of the conditions known to be important for optimum parasite development. These conditions include maintaining constant temperature of infected snails, systematic feeding, reduction of rotifer infestation as much as practical, etc. Fluctuations in cercarial and adult worm production persist even when controlling these known optimal conditions. It is important therefore to continue investigating details of the schistosome life cycle in order to supply materials in quantity for vaccine development.

SIGNIFICANT ACCOMPLISHMENTS

1) Established natural fluctuations of S. mansoni life cycle under our conditions

2) Compared the results of 1 miracidium vs 8-10 miracidia snail exposures in terms of:

   (a) patency of infection in snails

   (b) deaths of snails

   (c) cercarial production

   (d) cercarial infectivity

3) Demonstrated that rotifer-infestation of schistosome-infected snails reduced cercarial production.

Appendix 1.

List of Investigators Supplied with Schistosomal Materials on Contract ONR N00014-76-C-0146.

NAVAL MEDICAL RESEARCH INSTITUTE

Dr. W. E. Vannier
Dr. K. D. Murrell
Dr. D. A. Dean
Dr. A. H. Smith
Dr. P. Minard
Dr. D. W. Taylor
Dr. P. Coulis
Dr. V. Schinski
Dr. M. Stek
Dr. C. H. Dorsey
Dr. A. Attallah

BIOMEDICAL RESEARCH INSTITUTE

Dr. M. A. Stirewalt
Dr. F. A. Lewis
Dr. E. Hayunga
Dr. C. Cousin

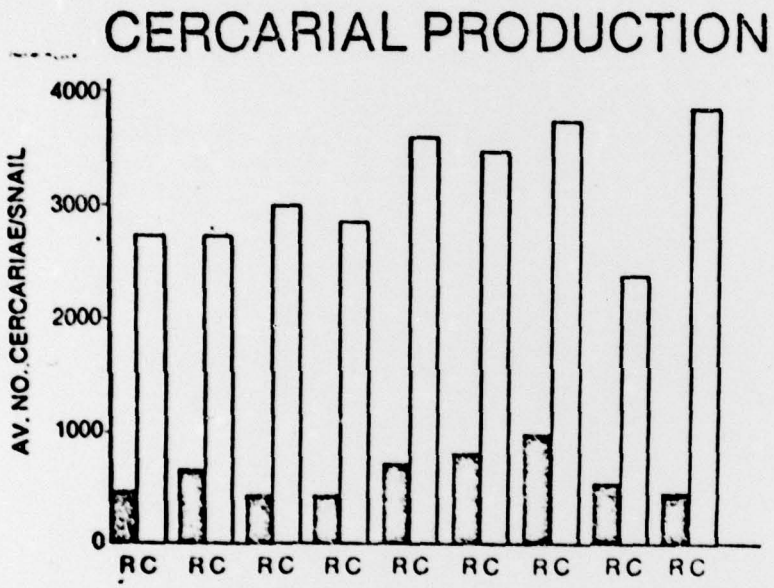


Figure 1. Histogram comparing the average number of cercariae per snail from Rotifer (R) and Non-Rotifer (C) Biomphalaria glabrata on nine consecutive Collecting Days.

CURRENT REPORTS AND PUBLICATIONS

SUPPORTED WHOLLY OR IN PART BY SCHISTOSOME MATERIALS

PRODUCED UNDER ONR CONTRACT No. N00014-76-C-0146

1978

Minard, P., Dean, D.A., Jacobson, R.H., Vannier, W.E., and Murrell, K.D. 1978. Immunization of mice with Cobalt-60 irradiated Schistosoma mansoni cercariae. Am. J. Trop. Med. & Hyg. 27: 76-86.

Minard, P., Dean, D.A., Vannier, W.E., and Murrell, K.D. 1978. Effect of immunization on migration of Schistosoma mansoni through lungs. Am. J. Trop. Med. Hyg. 27: 87-93.

Dean, D.A., Minard, P., Stirewalt, M.A., Vannier, W.E. and Murrell, K.D. 1978. Resistance of mice to secondary infection with Schistosoma mansoni. I. Comparison of bisexual and unisexual infections. Am. J. Trop. Med. Hyg. 27: 951-956.

Stirewalt, M.A. 1978. Quantitative collection and proteolytic activity of preacetabular gland enzyme(s) of cercariae of Schistosoma mansoni. Am. J. Trop. Med. & Hyg. 27: 548-553.

Dean, D.A., Minard, P., Murrell, K.D. and Vannier, W.E. 1978. Resistance of mice to secondary infection with Schistosoma mansoni. II. Evidence for a correlation between egg deposition and worm elimination. Am. J. Trop. Med. & Hyg. 27: 957-965.

Murrell, K.D., Taylor, D.W., Vannier, W.E. and Dean, D.A. 1978. Schistosoma mansoni: Analysis of surface membrane carbohydrates using lectins. Exp. Parasitol. 46: 247-255.

Attallah, A.M., Smith, A.H., Murrell, K.D., Fleischer, T., Woody, J., Vannier, W.E., Scher, I., Ahmed, A. and Sell, K.W. 1979. Characterization of immunosuppressive state during Schistosoma mansoni infection. J. Immunol. 122: 1413-1420.

Murrell, K.D., Stirewalt, M.A. and Lewis, F.A. 1979. Schistosoma mansoni: Vaccination of mice with cryopreserved irradiated schistosomules. Exp. Parasitol. (in press).

Stirewalt, M.A., Lewis, F.A. and Murrell, K.D. 1979. Schistosoma mansoni: Cryopreservation of schistosomules. Exp. Parasitol. (in press).

ABSTRACTS OF PRESENTATIONS

1978

Stirewalt, M., Murrell, K.D., and Lewis, F.A. Cryopreservation of schistosomules of Schistosoma mansoni. American Society of Tropical Medicine & Hygiene, 7-11 Nov 1978, Chicago, Ill.

Lewis, F.A., Campbell, D.L. and Stirewalt, M. Murine cellular reactivity to preacetabular gland contents of Schistosoma mansoni. American Society of Tropical Medicine & Hygiene, 7-11 Nov 1978, Chicago, Ill.

Cousin, C., Stirewalt, M. and Dorsey, C.H. Comparative transformation of in vivo and in vitro schistosomules, Program and Abstracts of the 52nd Annual Meeting of the American Society of Parasitologists, 6-11, Nov 1978, Chicago, Ill.

Stirewalt, M. and Lewis, F.A. Effect of rotifers on schistosome cercariae. Program and Abstracts of the 52nd Annual Meeting of the American Society of Parasitologists, 6-11 Nov 1978, Chicago, Ill.

Murrell, K.D., Clark, S.S., Weaver, N., Stirewalt, M., Vannier, W.E. and Dean, D.A. Immunization with attenuated Schistosoma mansoni schistosomules, Fourth International Congress of Parasitologists, Warsaw, Poland, 19-26 Aug. 1978.

Murrell, K.D., Minard, P., Vannier, W.E. and Dean, D.A. 1978. Immunization against schistosomes. Proc. Int'l Conference on Schistosomiasis, Cairo, Egypt, October 18-25, 1975, Vol. II, p. 545-555.

Dean, D.A. 1978. Studies on the mechanism of acquired immunity to Schistosoma mansoni. Int'l Conference on Schistosomiasis, Cairo,

Minard, P., Murrell, K.D. and Stirewalt, M.A. 1978. Penetration enzymes of Schistosoma mansoni cercariae. Proc. Int'l Conference on Schistosomiasis, Cairo, Egypt, October 18-25, 1975, Vol. II, p. 599-602.

Schinski, V.D., Clutter, W.G. and Murrell, K.D. 1978. Labelled antiglobulin assays for the study of schistosome antigens and human immune responses. Proc. Int'l Conference on Schistosomiasis, Cairo, Egypt, October 18-25, 1975, Vol. II, p. 583-585.

Vannier, W.E., Hussain, R. and Murrell, K.D. 1978. Schistosome allergens. Proc. Int'l Conference on Schistosomiasis, Cairo, Egypt, October 18-25, 1975, Vol. II, p. 587-596.